

~~Q~~ "The former, Serial No. 07/841,910, has been allowed, and the latter, Serial No. 07/886,660, issued as U.S. Patent No. 5,449,767 on September 12, 1995. Therefore, the disclosures of all three aforementioned U.S. Patent Nos. 4,711,955, 5,328,824 and 5,449,767 are herein incorporated by reference and made part of the instant disclosure."

and replace with the following:

~~Q~~ -- The former, Serial No. 07/841,910, was issued as U.S. Patent No. 5,476,928 on December 19, 1995, and the latter, Serial No. 07/886,660, was issued as U.S. Patent No. 5,449,767 on September 12, 1995. Therefore, the disclosure of all four aforementioned U.S. Patent Nos. 4,711,955, 5,328,824, 5,476,928 and 5,449,767 are herein incorporated by reference and made part of the instant disclosure. --

In The Claims:

Add new claims 337-372.

-- 337. (NEW) A process for preparing a labeled oligo- or polynucleotide of interest, comprising the steps of:

(A) providing:

~~Q~~ one or more chemically modified nucleotides capable of incorporating into an oligo- or polynucleotide, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, said other modified or unmodified nucleic acids being capable of incorporating into an oligo- or polynucleotide, said chemical modification comprising a label capable of providing directly or indirectly a detectable signal indicating the presence of said labeled oligo- or polynucleotide, said chemically modified nucleotides being modified on the sugar, phosphate or base moieties thereof and being selected from the group consisting of:

(i)

PM—SM—BASE—Sig

wherein

PM is a phosphate moiety,

SM is a sugar moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety, and

wherein PM is attached at the 3' or the 5' position of the sugar moiety SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE directly or through a linkage group at a position other than the C⁵ position when BASE is a pyrimidine, at a position other than the C⁸ position when BASE is a purine, and at a position other than the C⁷ position when BASE is a 7-deazapurine;

(ii)



wherein

PM is a phosphate moiety,

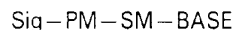
SM is a sugar moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety, and

wherein said PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group; and

(iii)



wherein

PM is a phosphate moiety,

SM is a sugar moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety; and

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wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is purine, and Sig is covalently attached to PM directly or through a linkage group; and

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(A) said oligo- or polynucleotide of interest; and
(B) incorporating said one or more modified nucleotides into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest. --

-- 338. (NEW) The process of claim 337, wherein said providing step Sig is covalently attached to BASE, SM or PM through a linkage group. --

-- 339. (NEW) The process of claim 338, wherein said linkage group contains an amine. --

-- 340. (NEW) The process of claim 339, wherein said amine comprises a primary amine. --

-- 341. (NEW) The process of claim 338, wherein said linkage group does not substantially interfere with formation of the signaling moiety or detection of the detectable signal. --

-- 342. (NEW) The process of claim 337, wherein said incorporating step is carried out using an enzyme. --

-- 343. (NEW) The process of claim 342, wherein said enzyme comprises a polymerase. --

-- 344. (NEW) The process of claim 343, wherein said polymerase comprises DNA polymerase. --

-- 345. (NEW) The process of claim 337, wherein said one or more chemically modified nucleotides or said other modified or unmodified nucleic acids comprise a nucleoside di- or tri-phosphate. --

-- 346. (NEW) The process of claim 337, wherein said incorporating step is template dependent or template independent. --

247 (NEW) The process of claim 346, wherein said incorporating step is template dependent. --

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-- 348. (NEW) A process for detecting the presence of an oligo- or polynucleotide of interest in a sequencing gel, comprising the steps of:

(A) providing:

(a) one or more chemically modified nucleotides capable of incorporating into an oligo- or polynucleotide, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, said other modified or unmodified nucleic acids being capable of incorporating into an oligo- or polynucleotide, said chemical modification rendering said one or more chemically modified nucleotides either:

(I) self-signaling or self-indicating or self-detecting; or

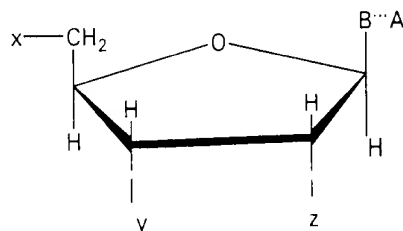
(II) comprising a label capable of providing directly or indirectly a detectable signal;

said self-signaling or self-indicating or self-detecting chemical modification or said label indicating the presence of said labeled oligo- or polynucleotide; thereby indicating the presence of said labeled oligo- or polynucleotide, said chemically modified nucleotides being modified non-disruptively or disruptively on at least one of the sugar, phosphate or base moieties thereof; and

(b) an oligo- or polynucleotide;

(B) incorporating said one or more chemically modified nucleotides into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest, said labeled oligo- or polynucleotide of interest comprising one or more chemically modified nucleotides selected from the group consisting of:

(i)

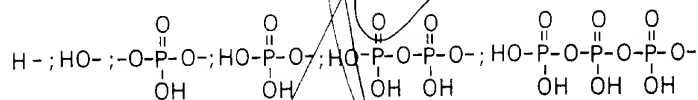


wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety covalently bonded to the C1'-position of the sugar moiety, provided that whenever B is a purine or 7-deazapurine, the sugar moiety is attached at the N9-position of the purine or 7-deazapurine, and whenever B is a pyrimidine, the sugar moiety is attached at the N1-position of the pyrimidine;

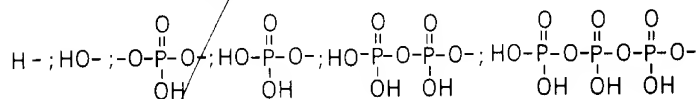
wherein A comprises at least three carbon atoms and represents at least one component of a signalling moiety capable of producing directly or indirectly a detectable signal or being self-signaling or self-indicating or self-detecting; and

wherein B and A are covalently attached directly or through a linkage group, and

wherein x comprises a member selected from the group consisting of:



wherein y comprises a member selected from the group consisting of:



wherein z comprises a member selected from the group consisting of
 H- and HO-;

(ii)

Sig

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PM-SM-BASE

wherein

PM is a phosphate moiety,

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SM is a sugar moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety, and

wherein said PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, said BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group; and
(iii)

Sig-PM-SM-BASE

wherein

PM is a phosphate moiety,

SM is a sugar moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety, and

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N¹ position when BASE is a pyrimidine or the N⁹ position when BASE is purine, and Sig is covalently attached to PM directly or through a linkage group; and

(b) said oligo- or polynucleotide of interest;

(C) transferring said labeled oligo- or polynucleotide of interest to a sequencing gel;

(D) separating said labeled oligo- or polynucleotide of interest from other nucleic acids not of interest; and

(E) detecting directly or indirectly the presence of said labeled oligo- or polynucleotide. --

-- 349. (NEW) The process of claim 348, wherein said incorporating step, A in the nucleotide (i) is covalently attached to B through a linkage group. --

- 350. (NEW) The process of claim 349, wherein said linkage group contains an amine. --
- 351. (NEW) The process of claim 350, wherein said amine comprises a primary amine. --
- 352. (NEW) The process of claim 348, wherein said incorporating step, Sig in the nucleotide (ii) is covalently attached to SM through a linkage group. --
- 353. (NEW) The process of claim 352, wherein said linkage group contains an amine. --
- 354. (NEW) The process of claim 353, wherein said amine comprises a primary amine. --
- 355. (NEW) The process of claim 348, wherein said incorporating step, Sig in the nucleotide (iii) is covalently attached to PM through a linkage group. --
- 356. (NEW) The process of claim 355, wherein said linkage group contains an amine. --
- 357. (NEW) The process of claim 356, wherein said amine comprises a primary amine. --
- 358. (NEW) The process of claims 349, 352 or 355, wherein said linkage group or groups do not substantially interfere with formation of the signaling moiety or detection of the detectable signal. --
- 359. (NEW) The process of claim 348, wherein said incorporating step is carried out using an enzyme. --
- 360. (NEW) The process of claim 359, wherein said enzyme comprises a polymerase. --
- 361. (NEW) The process of claim 360, wherein said polymerase comprises DNA polymerase. --

-- 362. (NEW) The process of claim 348, wherein said one or more chemically modified nucleotides or said other modified or unmodified nucleic acids comprise a nucleoside di- or triphosphate. --

-- 363. (NEW) The process of claim 348, wherein said incorporating step is template dependent or template independent. --

-- 364. (NEW) The process of claim 363, wherein said incorporating step is template dependent. --

-- 365. (NEW) The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide. --

-- 366. (NEW) The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one external modified nucleotide. --

-- 367. (NEW) The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one external modified nucleotide. --

-- 368. (NEW) The process of claim 348, wherein said separating step is carried out electrophoretically. --

-- 369. (NEW) The process of claim 349, wherein said detecting step is carried out directly. --

-- 370. (NEW) The process of claim 348, wherein said direct detection is carried out on one or more self-signaling or self-indicating or self-detecting nucleotides. --

-- 371. (NEW) The process of claim 370, wherein said one or more self-signaling or self-indicating or self-detecting nucleotides comprise fluoresceinated nucleotides. --

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Serial No.: 08/486,069
Filed: June 7, 1995
Page 10 [(Supplemental Amendment to Applicants' March 28, 1997 Amendment
Under 37 C.F.R. 51.115) - September 16, 1997]

-- 372. (NEW) The process of claim 371, wherein said fluoresceinated nucleotides
comprise fluoresceinated DNA. --

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